Histopathological Changes that Occur on the Testicular and Penile Tissues Depending on the Treatment of Human Chorionic Gonadotropin: Rat Model

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ABSTRACT

Objectives: To examine the histopathological effects of hCG treatment on the penile and the testicular tissue in rat model.

Materials and Methods: The rats of the hCG group (n = 8) were given daily subcutaneous injections of 50 IU of hCG for 15 days (Pregnyl, Organon). Rats of the control group(n = 8) received subcutaneous isotonic saline. All rats were sacrificed at the first month after hCG administration. After the received tissue samples were examined germinal epithelial cell thickness, seminiferous tubule diameter, internal diameter of the tubules, the number of germ cell layers in the testicular tissue and the diameters of penis, cavernous sinus lumen diameters and collagen tissue amount in the cavernous sinus surrounding were assessed in the sections prepared from the penis.

Results: İt was detected a decrease in the testis weight, a clear atrophy in the tubules, a reduction in spermatogenesis, a clear decrease in the mature spermatocytes, lower the mean thickness and the number of cell layers of the germinal membrane in testicular tissue in the hCG group. İt was found that the amount of collagen in penile tissue was higher in the hCG group and diameters of cavernosal sinus lumens, diameter of the penis was lower than in the hCG group

Conclusion: hCG led to the deterioration in testicular histology and the histological changes in the penile tissue. The degradation in the testicular tissue and these changes formed in the penile tissue may affect the erectile tissue function.

Keywords: Histopathology, Human Chorionic Gonadotropin, Penile, Rat, Testicular.

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INTRODUCTİON

Cryptorchidism affects 3–4% of all full-term male neonates, with the prevalence decreasing to 0.8% by 1 year of age.The prevalence of cryptorchidism is approximately 3–4% in all term male neonates, with the prevalence decreasing to 0.8% at 1 year of age.(1) It was revealed that 50% of unilateral cases and two thirds of bilateral cases are associated with eventual fertility problems.(2) Thus, studies to improve fertility have focused on earlier surgical intervention (3,4) and, more recently, hormonal therapy.(5) Hormonal treatment with human chorionic gonadotropin (hCG) may be given initially for cryptorchidism because of reported testicular descent in about 20% of cases.(6) Although some studies recommend early hormonal treatment for cryptorchidism (7,8) at 1500 IU hCG i.m. weekly for 3 weeks (9), others revealed that hormonal treatment may harm the germ cells[10].(10) It is still questionable whether early hormonal treatment is safe for germ cells or not.

hCG used in the treatment of cryptorchidism may cause the appearance of secondary sex characteristics such as early epiphyseal closure, pubic hair, genital growth, aggressive behavior and scrotal hyperpigmentation. However, there are no studies assessing the occurring of the genital growth. In children who had been treated with hCG, there have not been the studies examining the changes that occur in the penile tissue.

When we look at the literature, we examined these effects on the rat model since there are no studies examining the histopathological effects of hCG treatment on the penile tissue and the effects on the testicular tissue.

MATERİALS AND METHODS

Wistar albino rats weighing 250 ± 50 g were used in this experimental study. After the approval of the ethics committee of our institution (Yuzuncu Yıl University Ethics Committee, Van) had been obtained, the care and use of laboratory animals was followed. Sixteen 6 weeks old male Wistar albino rats were maintained under standard conditions of temperature and 12-hour day/night cycles with food and water ad libitum and a constant temperature of 20–22 ° C. They were given free access to tap water. Upon arrival, they were randomized to hCG and control groups (n = 8 each). The rats of the hCG group were given daily subcutaneous injections of 50 IU of hCG for 15 days (Pregnyl, Organon). Rats that served as the control group received subcutaneous isotonic saline (n = 8). All rats were sacrifi ced by a lethal overdose of sodium pentobarbital (100 mg/kg, i.p.) to obtain normal descended testicular and penile tissue at the first month after hCG administration. After the received tissue samples were detected in 10% buffered formalin, the 4μm-sections were taken with the microtome by being embedded into paraffin blocks. The sections were examined in the research microscope by being painted with hematoxylin-eosin stain.

Three sections (proximal, central and distal) were taken from each testis, and in each section, 10 seminiferous tubules were chosen for analysis (i.e. 30 tubules per testis). Spherical or slightly elliptical tubules with the lumen centrally located were chosen because this signified a perpendicular cross-section that would most accurately depict germinal epithelial cell thickness and architecture. Germinal epithelial cell layer thickness was determined by counting the number of epithelial cells from the basement membrane to the lumen at 90, 180, 270 and 360 ° , and averaging. The seminiferous tubule diameter (STD), the internal diameter of the tubules, and the diameter and the number of germ cell layers were measured. The diameters of penis, cavernous sinus lumen diameters and collagen tissue amount in the

cavernous sinus surrounding were assessed in the sections prepared from the penis. Data were analysed by the Mann–Whitney U -test with $P < 0.05$ considered to indicate significance.

RESULTS

When compared with the control group, a decrease was detected in the testis weight in the hCG group (Table 1). When the weight difference was histopathologically examined, a clear atrophy was detected in the tubules. In addition, a reduction in spermatogenesis and a clear decrease in the mature spermatocytes were detected (Figure 1). Consequently; the mean thickness and the number of cell layers of the germinal membrane in testicular tissue of the hCG group were significantly lower than those of the control group ($p < 0.05$) (Figure 2). İt was found that the percentage of the open seminiferous tubular lumen in testicular tissue of all hCG treated rats was significantly higher than that of the control group ($p < 0.05$). We could find statistical difference between mean seminiferous tubular diameter in testicular tissues of hCG treated rats was significantly higher than that of the control group ($p < 0.05$).

At the first month after hCG administration for 15 days, it was found that the amount of collagen in penile tissue of all hCG treated rats was significantly higher than that of the control group ($p < 0.05$). Additionally, we were found diameters of cavernosal sinus lumens (Figure 3A,B) and diameter of the penis (Table 1) of all hCG treated rats was significantly lower than that of the control group ($p < 0.05$).

DİSCUSSİONS

Cryptorchidism is the most common disorder of sexual differentiation in man.(11) While 89% of patients with untreated bilateral cryptorchidism develop azoospermia, only 32% of those

treated with gonadotrophins do so.(12) Hormonal treatment of cryptorchidism was introduced 84 years ago and the first hormone used was hCG.(13) hCG is a polypeptide hormone produced by the human placenta, with an α -subunit that is almost identical to that of FSH, thyroid-stimulating hormone and LH, so its action is similar to that of LH. A meta-analysis of 4524 cases of cryptorchid testes showed that hCG treatment has a success rate of 19% compared to a placebo effect of 4% .(6)

Recently, Schwentner et al. showed that neoadjuvant hormonal treatment with gonadotropin-releasing hormone improves the fertility index in prepubertal cryptorchidism.(14) In contrast to these studies, it was shown that histological findings indicated a reduction in the number and maturation of germ cells in cryptorchid infants treated with combined hormonal therapy.(7) Cortes et al. reported that hormonal treatment given for testicular descent may harm the germ cells.(10) It was also reported that unsuccessful hCG treatment resulted in lower spermatogonia per tubule in boys with cryptorchidism.

In the rat testis, germ cell death occurs through apoptosis, characterized by internucleosomal fragmentation of DNA, and this is regulated by androgens and gonadotrophins. In cryptorchid boys, germ cell apoptosis was assessed after unsuccessful hCG treatment at simultaneous orchidopexy and testicular biopsy. There were more apoptotic germ cells in the contralateral scrotal than in the undescended testis; both interstitial cells and germ cells were affected, and the germ cells undergoing apoptosis were exclusively spermatogonia. Germ cell apoptosis was increased in both scrotal and inguinal testes, returning to the initial level 1 month after the hCG treatment, suggesting that hCG withdrawal increases germ cell apoptosis in the human testis.(15)

Because of increasing serum testosterone levels during hCG treatment by about 150–200 fold, followed by a decrease to low prepubertal levels within a few weeks, it was assumed that the increase in germ cell apoptosis after hCG treatment most likely refl ects the androgen

withdrawal effect. Thus, normal development of the testis is disrupted by the hCG treatment, possibly through the mechanism of increased apoptotic germ cell death. Consequently, it was proposed that because of poor treatment success rates and shown side effects, the potential hazards of hCG treatment on the testis should be critically reevaluated.(16)

The rat model appears particularly useful in a study evaluating testicular histology, because developmental parameters in rats are similar to those in humans. Kaya et al. showed that gonadotropin gonadotropin therapy deteriorated the seminiferous tubular histology of testes in rats.(17) However, their results showing detrimental effects of hCG do not conflict with those of previous series.(10,15,16) The mean percentage of the open seminiferous tubular lumen in testicular tissues of all hCG-treated rats was found to be significantly higher than that of control rats . Similarly, mean thickness and the number of cell layers of the germinal membrane were signifi cantly lower in testicular tissues of all hCG groups at the first month. In a study conducted by Karaman et al they reported that these histological changes that the hCG made in the testicles were reversible and dose dependent.(18)

When looked at the literature, the number of the studies showing the effect of hCG on the normal testis tissue is limited and also the results are controversial. When compared to the control group, we found the decline in testicular weight in the hCG group. When the weight difference was histopathologically examined, a clear atrophy was detected in the tubules and connected to it. In addition, a reduction in spermatogenesis and a clear decrease in the mature spermatocytes were detected. Accordingly; the mean thickness and the number of cell layers of the germinal membrane in testicular tissue of the hCG group were significantly lower than those of the control group ($p < 0.05$). We could find statistical difference between mean seminiferous tubular diameter in testicular tissues of hCG treated rats was significantly higher than that of the control group ($p < 0.05$). The percentage of the open seminiferous tubular lumen in testicular tissue of all hCG treated rats was significantly higher than that of

the control group. According to all of our findings, we observed that Hcg had the negative effects on the testicular tissue. Our findings related to the histological changes formed in rat testis due to the use of hCG have supported the findings of Kaya et al (17)

When we look at the literature, there is no study simultaneously examining the effect on the penile tissue of hCG and with its effects on testis-penis in rat the model. In our study, at the first month after hCG administration for 15 days, it was found that the amount of collagen in penile tissue of all hCG treated rats was significantly higher than that of the control group ($p < 0.05$). Additionally, diameters of cavernosal sinus lumens and diameter of the penis of all hCG treated rats were found significantly lower than that of the control group ($p < 0.05$). It was observed that there were the collagen tissue growth around the cavernous sinus and accordingly the contraction the lumens and as a result the shrinkage in the diameter of the penis. These results make us think that the erectile tissue function changes may become due to the use of hCG and it may affect the erectile function during the puberty.

In this study; we saw that hCG led to the deterioration in testicular histology and the histological changes in the penile tissue. In order to be able to clearly assess the impact on erectile tissue function of the degradation in the testicular tissue and these changes formed in the penis tissue, it is needed much more long-term and further studies, including the biochemical parameters in together.

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Table 1: Testes weight and measurement of penis diameters

	hCG group (n 8)	Control group(n 8)	P value
Testes weight(g)	4,375	5,425	$p < 0.05$
Penis diameters (μm)	3467	3818	$p < 0.05$

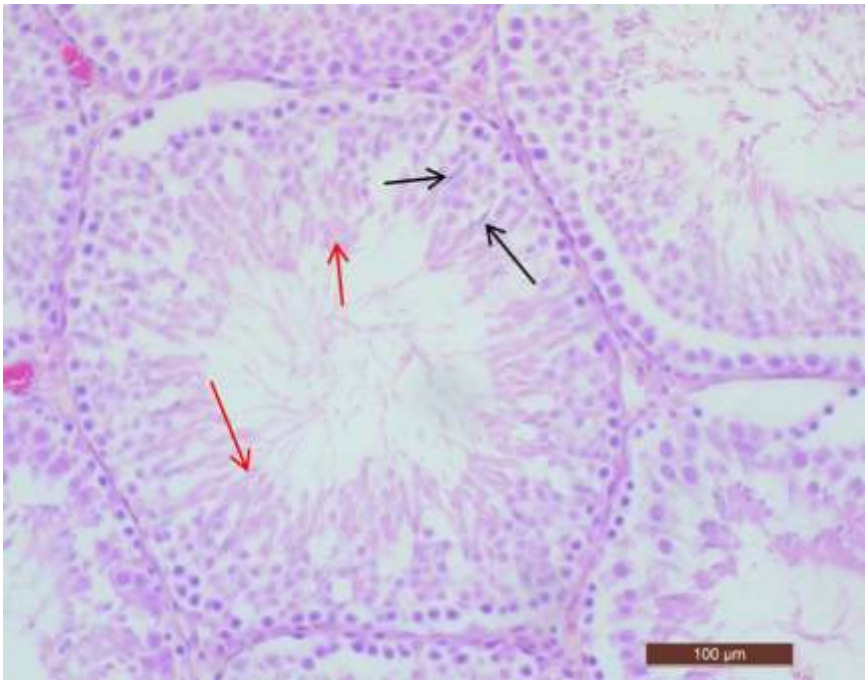

Fig. 1: Decreased spermatits in hCG group testes (black arrows), degenerated spermatogoniums (red arrows) (HxE) Bar =100uM.

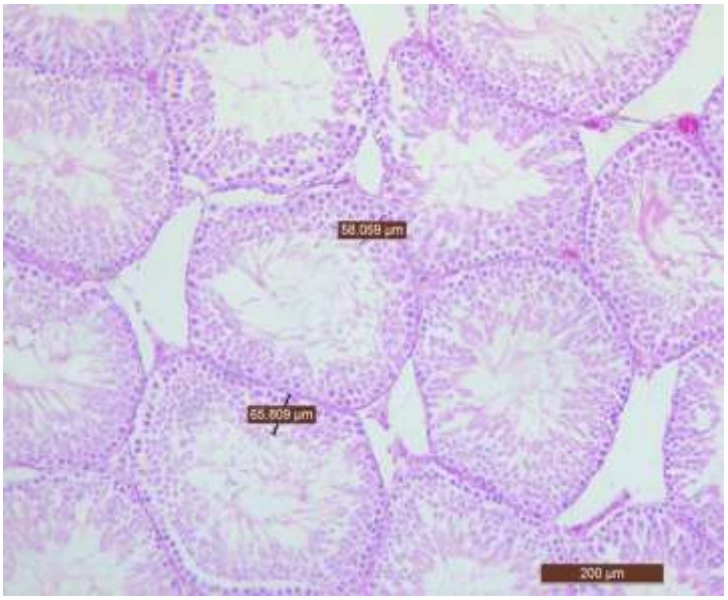

Fig. 2: Germinal membrane thickness in the testicular tubules in hCG group (HxE) Bar =200µm.

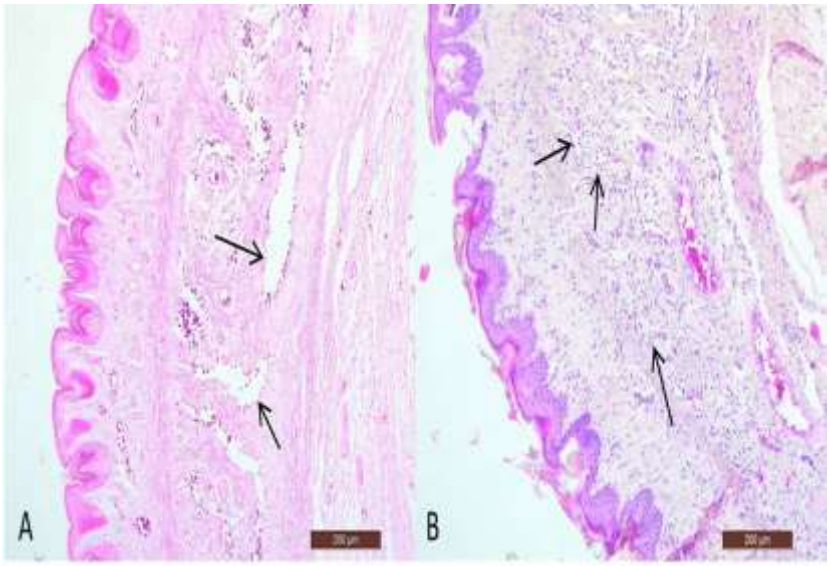

Fig. 3: A: Cavernous sinuses in healthy penis in the control group (arrows) (HxE) Bar = 200µm B: Narrowed cavernous sinuses in hCG group penis (arrows) (HxE) Bar =200µm.